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Microcosm Evaluation of the Fate, Toxicity, and Risk to Aquatic Macrophytes from Perfluorooctanoic Acid (PFOA)

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Abstract. Perfluorooctanoic acid (PFOA) is an anthropogenic contaminant detected in various environmental and biological matrices. This compound is a fluorinated surfactant, belonging to a class of molecules known for persistence and their global distribution, but for which little ecotoxicological data are currently available, especially under field conditions. The environmental fate and toxicity of PFOA to the aquatic macrophytes *Myriophyllum sibiricum* and *M. spicatum* were investigated using 12,000 L outdoor microcosms. Replicate microcosms ($n = 3$) were treated with 0.3, 1, 30, and 100 mg/L PFOA as the sodium salt, plus controls, and assessed at regular intervals over 35 days. PFOA showed no significant dissipation from the water column, except at the greatest concentration, where partitioning from the water column into other compartments is suspected. The two species of *Myriophyllum* were similar in their sensitivity to PFOA under these simulated field conditions. Toxicity after 14 to 35 days of exposure in the evaluated endpoints for *M. spicatum* was ≥5.7 mg/L PFOA for EC_{10}s and ≥31.8 mg/L PFOA for EC_{50}s and in *M. sibiricum* was ≥8.4 mg/L PFOA for EC_{10}s and ≥35.8 mg/L PFOA for EC_{50}s. The no observed effects concentrations (NOECs) for *Myriophyllum* spp. were consistently ≥23.9 mg/L PFOA. A risk assessment for these plant species estimated a negligible probability of toxicity being observed from PFOA exposure at current environmental concentrations.

Perfluorinated surfactants are a class of compounds with numerous industrial and consumer applications (Gillian and Mandel 1993; Key *et al.* 1997). Perfluorooctanoic acid (PFOA) is just one of several compounds with the unique fluorine chemistry that allows the compound to repel both water and lipid compounds. These compounds have attracted attention due to their extreme stability under a variety of environmental conditions and their widespread distribution in various environmental and biotic matrices (Welter 1979; Giesy and Kannan 2001; Kannan *et al.* 2001, 2002; Hansen *et al.* 2002; Moody *et al.* 2002; Moriwaki *et al.* 2003; Sissel 2003; Martin *et al.* 2004). There are a number of possible sources of PFOA introduction into the aquatic environment, including firefighting foams, combustion of fluoropolymers such as Teflon, precursor compounds such as fluorotelomer alcohols, and releases from production facilities with environmental concentrations usually in the ng/L to low μg/L range (Moody and Field 1999, Moody *et al.* 2002; Ellis *et al.* 2001, 2003, 2004; Hansen *et al.* 2002; Dinglasan *et al.* 2004). No environmental half-lives under field conditions have been reported to date in the scientific literature to the knowledge of the authors. Because degradation of PFOA and similar fluorinated surfactants has not been observed under normal environmental conditions, concentrations could increase with continued production and inputs into the environment (Rembe and Debus 1996). This will be mitigated by the fact that 3M, a manufacturer of PFOA, phased out production by the end of 2003, but Dupont continues to manufacture this compound (Brown and Mayer 2000).

Some work has been conducted to address the potential aquatic toxicity associated with this compound, but much of it has focused on invertebrates. Studies with indoor microcosms found *Daphnia magna* to be the most sensitive of a number of invertebrate species, with lowest observed effect concentrations of 10 to 70 mg/L PFOA within 1 to 7 days of initial exposure (Sanderson *et al.* 2003). *Rotifera* spp. were observed to be the least sensitive invertebrate assemblage in the test system. The laboratory 48-hr LC_{50} for *D. magna* has been reported as 632 mg/L, and the 14-day EC_{50} for the green algae, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) has been reported at 73 mg/L (3M 2000). The EC_{10}s and EC_{50}s for a variety of endpoints for *Myriophyllum spicatum* exposed to perfluorooctane sulfonic acid as the potassium salt (PFOS) in the same test systems were >3 mg/L and >12 mg/L PFOS, respectively, while the EC_{10}s and EC_{50}s

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for *M. sibiricum* were >0.1 mg/L and >1.6 mg/L PFOS, respectively (Hanson *et al.* 2004). In order to properly address the potential risks PFOA poses to aquatic systems, toxicological data from a range of aquatic organisms are required.

This study was part of a larger series of investigations into the fate and effect of fluorinated surfactants on aquatic ecosystems (Boudreau *et al.* 2003a, 2003b; Sanderson *et al.* 2002, 2003, 2004; Hanson *et al.* 2004). In this study we evaluated the toxicity of PFOA to the aquatic macrophytes *Myriophyllum sibiricum* and *M. spicatum* under seminatural field conditions using outdoor microcosms. We also evaluated the environmental fate of PFOA in the microcosm water and conducted a risk assessment for these plants and PFOA based on the results generated from this study.

Materials and Methods

The Microcosms

The University of Guelph Microcosm Facility, located at the Guelph Turfgrass Institute, Ontario, Canada, consists of 30 microcosms. The microcosms are approximately 1.2 m deep with a water depth of 1 m, a diameter of 3.9 m, and a surface area of 11.95 m^2, and each has a capacity of approximately 12 m^3 of water. The microcosms are below ground with the tops flush with the surface. Sediment trays filled with amended sediment (Evergreen Sod Company, Waterdown, Ontario, Canada) were placed in the bottom of each microcosm covering approximately 50% of the available surface area. The sediment consisted of a 1:1:1 mixture of sand, loam, and organic matter (mainly composted manure). The total carbon content of the sediment was 16.3%, with inorganic carbon comprising 2.4% and the organic fraction being 13.9% as determined by combustion in a Leco CR12 Carbon Analyzer (Leco, St. Joseph, MI). Prior to the initiation of the study, the microcosms were circulated for 2 weeks from a well-fed irrigation pond. Detailed descriptions of the microcosms used in this study are provided in Hanson *et al.* (2001).

Water Chemistry and PAR

Water chemistry was monitored on a regular basis. Maximum and minimum temperatures and dissolved oxygen (DO) measurements (YSI model 57 meter, Yellow Springs, OH) were taken daily during the course of the study. On sampling days for water residue analysis for PFOA, water hardness, alkalinity, and pH were also measured. Water hardness and alkalinity were determined using standard methods and kits by Hach (Hach Company, Loveland, CO). Routine water chemistry determinations were taken at day −1, and at 1 hr, and 2, 4, 7, 14, 21, 28, and 35 days posttreatment.

Measurements of photosynthetically active radiation (PAR) were taken at regular intervals during the course of the study on clear sunny days, between 12 noon and 2 pm when sunlight is at its maximum intensity. The measurements were taken as close to the actual sampling date as possible, weather permitting. Readings were taken on a Li-Cor Quantum/Radiometer/Photometer Model LI-185A (Li-Cor, Lincoln, NE). A reading was taken at the surface and at a depth of 60 cm in each microcosm.

Treatment and Sampling Regime

The treatments applied to the microcosms were 0.3, 1, 30, and 100 mg/L PFOA, as the sodium salt, plus controls. The nominal concentrations applied were for the anion form of PFOA. Each exposure was randomly assigned to three separate microcosms ($n = 3$) from a total of 15 microcosms. The PFOA, donated by 3M (St. Paul, MN), was weighed out and suspended in irrigation pond water. Application of the PFOA to the microcosms took place on June 14, 2000 as previously described (Boudreau *et al.* 2003b). Immediately prior to treatment, water flow into each microcosm from the main irrigation pond ceased, creating a closed system relative to the other microcosms and the irrigation pond.

Water samples for PFOA analysis were taken at the same time as routine water chemistry samples. A metal integrated water column sampler (Solomon *et al.* 1982) was used to collect the water samples for residue analysis. Integrated subsamples from a minimum of four randomly selected locations in each microcosm were collected to a total volume of 4 L. A 125-ml aliquot of this sample was decanted into a Nalgene container (VWR, ON, Canada) for PFOA analysis, and the samples were stored at 4°C until analysis.

PFOA Residue Analysis

Water samples from the microcosms were analyzed for PFOA by ion chromatography. The mobile phase was 0.5 mM NaOH, 5% methanol, and 5% acetonitrile with a flow rate of 0.4 ml/min. The injection volumes varied from 5, 10, 75, and 200 µl for the 100, 30, 1, and 0.3 mg/L microcosms, respectively. A Perkin Elmer Series 200 HPLC pump with an Alltech ERIS 1000 suppressor and Alltech conductivity detector was used for the analysis. The column used was a Waters C-8 silica/polymeric hybrid guard column (2-cm length, 3.9-mm diameter). For each set of samples analyzed five standards and one quality control sample were included at the beginning of each run and again at the end.

Myriophyllum *spp. Experimental Design*

The basic experimental design has been described in detail elsewhere (Hanson *et al.* 2001, 2002). Apical shoots (4 cm), free of lateral shoots, of laboratory-cultured *M. spicatum* and *M. sibiricum* (American Society for Testing and Materials 1999) were transferred to planting trays containing the same sediment as the microcosms 1 day prior to the treatment of the microcosms. The plants were placed in the microcosms on June 13, 2000.

Plants were assessed 1 day prior to treatment with PFOA, and at 7, 14, 21, and 35 days posttreatment. The final day of the *Myriophyllum* spp. study, day 35, was July 18, 2000. At each sampling interval, two plants of each species were removed and evaluated, except for day −1. On day −1, 10 plants of each species from the laboratory culture were evaluated as 4-cm apical shoots for the endpoints described below. On the other sampling days, plants were removed randomly from the microcosms and transported back to the laboratory in their respective microcosm water for immediate analysis of the endpoints listed below.

A number of endpoints were monitored over the course of the study. These were growth (plant length), biomass (wet mass/dry mass), root number (primary roots from the plant stem), primary root lengths (total and longest), and number of nodes. Pigment concentrations, namely chlorophyll a/b and carotenoid content, were also assessed. Chlorophyll a/b and carotenoid concentrations were determined according to standard methods (American Society for Testing and Materials 1999) on a Beckman DU-65 Spectrophotometer (Beckman Coulter, Fullerton, CA).

Statistical Analysis

Data from toxicity testing with both plant species were evaluated using nonlinear regression techniques described in Stephenson *et al.*

Table 1. Reparameterized equations used to fit the concentration-responses of perfluorooctanoic acid–exposed *Myriophyllum sibiricum* and *M. spicatum* in SAS version 8.2

Regression	Equation[a]
Linear	$y = ((-b \times x/100)/ EC_x) \times C_0 + b$
Logistic	$y = a/[1 + (x/ (100-x) (C_0 / EC_x)^b)]$
Gompertz	$y = g \times \exp ((\log ((100-x)/100) \times (C_0 / EC_x)^b)$
Exponential	$y = a \times \exp (\ln ((a-a \times x/100-b \times (100-x)/100)/a) \times (C_0 / EC_x)) + b$
Hormetic	$y = (t \times (1 + h \times C_0))/ (1 + ((x/100 + h \times (100-x)/100))/0.5) \times (C_0 /EC_x)^b)$

[a] The variable EC_x (i.e., EC_{10}) is the calculated effective concentration at which x percent (i.e., 10%) of the endpoint is affected, C_0 is the actual concentration (i.e., mg/L), y is the response or change from control of the endpoint modeled, and a, b, g, t, and h are constants.

Table 2. The initial concentration, percent of nominal, and time-weighted averages for perfluorooctanoic acid (PFOA) applied to field microcosms in the anion form[a]

Nominal concentration of PFOA (mg/L)	Initial concentration of PFOA (mg/L)	Percent of nominal	Time weighted average
0.3	0.28 (0.02)	95	0.27 (0.03)
1	0.64 (0.07)	64	0.65 (0.02)
30	20.9 (7.1)	70	23.9 (1.5)
100	87.8 (7.5)	88	74.1 (3.7)

[a] The values in parentheses are the standard deviation of the mean of the three microcosm values for that concentration at 1 hr or for the 35-day time-weighted average.

(2000) with SAS Version 8.2 (SAS Institute, Cary, NC). Only new growth from the time of introduction into the microcosms (*e.g.*, shoot length, wet/dry mass, nodes) was used in the models so that a more sensitive and conservative estimate of toxicity was obtained. A total of five possible models were tested for best fit according to the criteria of Stephenson *et al.* (2000). The models tested were logistic, linear, exponential, hormetic, and Gompertz (Table 1). Effect measures were calculated at two levels of response, EC_{10} and EC_{50}, for each species, endpoint, and time point when possible. Because of the loss of a set of control plants, the controls for both species of *Myriophyllum* had an n of 2.

The no observed effects concentrations (NOECs) and lowest observed effect concentrations (LOECs) were calculated with a one-way analysis of variance ($p < 0.05$) in a completely randomized design in SAS Version 8.2 using General Linear Models with no adjustments for new growth. If assumptions of normality and equal variance were not met, the data were either ln or square root transformed. If data could not be effectively transformed, a non-parametric test, the Kruskal-Wallis on ranks, was conducted. When significance was found, treatments were compared to controls with a two-tailed Dunnett's test (% = 0.05). The time-weighted averages (Table 2) were used in place of the nominal concentrations for all statistical analyses.

Risk Assessment

The threshold of toxicity for PFOA to *M. sibiricum* and *M. spicatum* after various durations of exposure were calculated according to Hanson and Solomon (2002). The calculated EC_{10}s and EC_{50}s for each of the three dates evaluated with *Myriophyllum* spp. were plotted as a cumulative frequency distribution using a probability scale on the y-axis as a function of the $\log_{10}$ concentration. Plotting positions were expressed as percentages and calculated from the Weibull formula:

$$100 \times i/(n + 1) \quad (1)$$

where i is the rank of the datum and n is the total number of data points in the data set (Parkhurst *et al.* 1995). Data were plotted and linear regressions on the transformed data were calculated using SigmaPlot 5 (Jandel, San Rafael, CA). EC_xs that were beyond the highest concentration tested were not included in the calculation of the distributions. The toxicity threshold was defined as the concentration equivalent to the 0.1 centile of the distribution (Hanson and Solomon 2002). These distributions were then compared to an exposure distribution of PFOA from the Tennessee River, USA, downstream of a fluorinated surfactant manufacturing plant (Hansen *et al.* 2002) using an exceedence profile (Solomon *et al.* 2000).

The toxicity thresholds calculated from the EC_{10} effect measure distributions were used in a hazard quotient (HQ) approach to assess the risk to these plants from PFOA under field conditions. The HQ was calculated as:

$$HQ = EEC = /TBC \quad (2)$$

where TBC is the Toxicological Benchmark Concentration (*i.e.*, the toxicity threshold as calculated from the distributions) and EEC is the highest expected environmental concentration. Values greater than 1 indicate a potential for toxic effects to occur, and values of less than 1 indicate that toxicity is not likely to occur (Suter 1995), although there can be more rigorous interpretations depending on whether or not the test is chronic or acute (Touart 1995). This HQ was then compared to a HQ calculated for each plant species using the greatest measured environmental concentration for different locations and the most sensitive endpoint from PFOA toxicity at the EC_{10} level as the TBC in place of the NOEC (Hanson and Solomon 2002). Exposure concentrations for the freshwater aquatic environments were taken from the literature (Hansen *et al.* 2002; Moody *et al.* 2002, 2003). No uncertainty factor was needed for the effect data because it was based on field or model ecosystem toxicity (Forbes and Calow 2002), but an uncertainty factor of 10 was applied to the exposure data due to the persistence of PFOA.

Results

General Microcosm Parameters

The pH, PAR, max/min temperature, DO, hardness and alkalinity profiles of the microcosms over the course of the study

Table 3. Mean values for chemical/physical parameters of the microcosms averaged over the 35-day study; the number of measurement events (n) and standard deviation, in brackets, are also given*

PFOA (mg/L)	Maximum Temperature °C (n = 29)	Minimum Temperature °C (n = 29)	pH (n = 9)	DO (mg/L)[a] (n = 25)	Alkalinity (mg/L)[b] (n = 9)	Hardness (mg/L)[b] (n = 9)	PAR ($\mu E\ m^{-2}\ sec^{-1}$)[c] (n = 5)
control	21.5 (1.9)	17.9 (2.1)	8.3 (0.3)	7.3 (2.1)	133 (22)	218 (7)	459 (56)
0.3	21.9 (2.2)	18.1 (2.0)	8.4 (0.3)	7.6 (2.0)	133 (21)	218 (7)	481 (109)
1	21.5 (2.0)	17.8 (1.9)	8.5 (0.4)	7.5 (2.2)	132 (19)	217 (8)	387 (114)
30	22.0 (2.1)	18.3 (1.8)	8.5 (0.3)	7.5 (2.3)	131 (21)	217 (7)	514 (44)
100	21.8 (2.0)	17.9 (1.9)	8.7 (0.2)	8.5 (1.6)	131 (20)	218 (7)	376 (81)

*Measurements were taken regularly over the 35-day study period. At each measurement event, the mean for each treatment was calculated. These means were then averaged for all measurements at that treatment.

[a] Dissolved oxygen.

[b] Measured as mg/L $CaCO_3$.

[c] Photosynthetically active radiation, measured at a depth of 60 cm.

showed no significant differences between treatments (Table 3).

PFOA Fate

There were no changes in the concentration of PFOA at the 0.3, 1, and 30 mg/L treatments over the 35-day field study (Figure 1). However, the water column concentration of PFOA in the 100 mg/L treatments decreased by 32% over the 35-day study (Figure 1). Time-weighted average concentrations were calculated for the 35 day field study (Table 2). With the exception of the smallest concentration, which was 95% of the nominal value, all the exposure levels were 12% to 36% lower than their expected initial (t = 1 hr) nominal concentration (Table 2).

Myriophyllum *spp. Toxicity*

No statistically significant differences or concentration–response trends were noted after 7 days of exposure to PFOA in both species, although responses were observed in some endpoints at the greatest exposure concentration relative to controls, especially in plant mass and root measures. The NOECs for *Myriophyllum* spp. after 14 to 35 days of exposure were consistently ≥23.9 mg/L PFOA for all endpoints and therefore LOEC would be ≥74.1 mg/L PFOA at all time points, based on the next highest tested concentration (Tables 4 and 5). Regression analysis found most of the concentration–response relationships from 14 days of exposure onwards to be logistic or linear in form (Tables 4 and 5, Figures 2 and 3). Toxicity was observed in the assessed endpoints at concentrations >5.7 mg/L PFOA at the EC_{10} and >31.8 mg/L PFOA at the EC_{50} for *M. spicatum*. Toxicity was observed after 14 to 35 days of exposure at concentrations >8.4 mg/L PFOA at the EC_{10} and at >35.8 mg/L PFOA at the EC_{50} for *M. sibiricum*. The NOECs for the *Myriophyllum* spp. were on average (± standard deviation) 3.4 ± 1.8-, 2.6 ± 2.1-, and 2.0 ± 1.1-fold greater than the EC_{10}s on days 14, 21, and 35, respectively, and hence less conservative. Comparing the same endpoints at the EC_{50} for the two plant species, *M. sibiricum* was found to be 2.6-, 1.1-, and 1.1-fold more sensitive than *M. spicatum* after 14, 21, and 35 days of evaluation. Endpoint sensitivity varied depending on the plant species and date of evaluation, as well as the effect level chosen. Pigment levels were consistently the least sensitive endpoint for all evaluation dates and both plant species, and dry mass generally one of the most sensitive endpoints.

Risk Assessment

The thresholds of toxicity derived from the effect measure distributions for *M. spicatum*, *M. sibiricum*, and those for a combined *Myriophyllum* spp. distribution were consistent between plants and evaluation dates, in that the calculated threshold values were very similar (Table 6). An exposure distribution for PFOA from the Tennessee River, USA, showed that the likelihood of exceeding the toxicity threshold

Table 4. Effective concentrations required to cause a decrease in an endpoint by 10% and 50% from control (EC_{10}s and EC_{50}s) as calculated using linear or nonlinear regression, with associated 95% confidence intervals, as well as the no observable effect concentrations (NOECs) for *Myriophyllum spicatum* exposed to perfluorooctanoic acid as the sodium salt (PFOA) in aquatic microcosms

Endpoint	Time	EC_{10} (95% CI)	EC_{50} (95% CI)	Model	Parameters	r^{2a}	NOEC
Plant length (cm)	14	24.2 (0, 52.0)	121.2 (0, 260.1)	Linear	b = 18.08 EC_{10} = 24.25	0.20	74.1
Plant length (cm)	21	5.7 (0, 18.3)	31.8 (9.9, 53.8)	Logistic	a = 43.05 EC_{10} = 5.68 b = 1.27	0.80	23.9
Plant length (cm)	35	31.5 (0, 68.2)	52.8 (26.0, 79.6)	Logistic	a = 66.08 EC_{10} = 31.52 b = 4.26	0.90	23.9
Root number	14	51.6 (0, 134.1)	258.1 (0, 670.0)	Linear	b = 7.75 EC_{10} = 51.62	0.13	74.1
Root number	21	16.1 (5.8, 26.4)	80.5 (29.1, 131.9)	Linear	b = 12.67 EC_{10} = 16.01	0.43	23.9
Root number	35	10.2 (6.6, 13.7)	51.0 (33.2, 68.7)	Linear	b = 21.1 EC_{10} 10.19	0.70	23.9*
Root length (cm)	14	18.1 (5.1, 31.1)	90.5 (25.3, 155.6)	Linear	b = 49.10 EC_{10} = 18.09	0.38	74.1
Root length (cm)	21	11.4 (4.4, 18.4)	56.9 (22.1, 91.8)	Linear	b = 89.18 EC_{10} = 11.39	0.43	23.9
Root length (cm)	35	8.8 (5.9, 11.7)	43.9 (29.4, 58.4)	Linear	b = 220.90 EC_{10} = 87.78	0.71	23.9
Longest root (cm)	14	19.7 (11.3, 28.1)	98.3 (56.3, 140.3)	Linear	b = 12.3 x = 19.65	0.64	23.9*
Longest root (cm)	21	13.9 (8.5, 19.2)	69.3 (42.5, 96.2)	Linear	b = 16.20 EC_{10} = 13.87	0.67	23.9
Longest root (cm)	35	24.3 (0, 56.7)	62.7 (41.1, 84.3)	Gompertz	g = 21.12 EC_{10} = 24.27 b = 1.99	0.77	23.9
Node number	14	13.2 (2.4, 24.0)	66.1 (12.2, 120.0)	Linear	b = 5.91 EC_{10} = 13.22	0.31	74.1
Node number	21	21.8 (3.1, 40.4)	44.8 (23.7, 65.9)	Logistic	a = 8.41 EC_{10} = 21.76 b = 3.04	0.83	23.9
Node number	35	8.3 (5.3, 11.4)	41.7 (26.5, 56.8)	Linear	b = 16.19 EC_{10} = 8.33	0.67	23.9
Wet mass (g)	14	33.7 (0, 112.2)	112.8 (0, 267.2)	Logistic	a = 460.80 EC_{10} = 33.67 b = 1.82	0.37	74.1
Wet mass (g)	21	10.9 (0, 29.1)	37.3 (10.7, 63.9)	Logistic	a = 1307.50 EC_{10} 10.85 b = 1.78	0.69	23.9
Wet mass (g)	35	22.8 (0, 53.5)	38.7 (0, 82.2)	Logistic	a = 5167.40 EC_{10} = 22.82 b = 4.16	0.61	74.1
Dry mass (g)	14	18.1 (0, 37.4)	90.3 (0, 186.9)	Linear	b = 30.72 EC_{10} = 18.06	0.22	74.1
Dry mass (g)	21	13.5 (0, 34.9)	40.2 (10.2, 70.1)	Logistic	a = 65.15 EC_{10} = 13.49 b = 2.01	0.65	23.9
Dry mass (g)	35	19.7 (0, 53.5)	33.5 (0, 92.6)	Logistic	a = 380.70 EC_{10} = 19.67 b = 4.13	0.40	74.1
Chlorophyll-a	14	nc[b]	nc	nc	nc	nc	74.1
Chlorophyll-a	21	nc	nc	nc	nc	nc	74.1
Chlorophyll-a	35	22.1 (7.9, 36.4)	110.4 (38.9, 181.8)	Linear	b = 0.48 EC_{10} = 22.08	0.44	74.1
Chlorophyll-b	14	nc	nc	nc	nc	nc	74.1
Chlorophyll-b	21	nc	nc	nc	nc	nc	74.1
Chlorophyll-b	35	31.7 (0, 114.0)	117.9 (0, 308.4)	Logistic	a = 0.18 EC_{10} = 31.66 b = 1.67	0.32	74.1
Carotenoids	14	nc	nc	nc	nc	nc	74.1
Carotenoids	21	nc	nc	nc	nc	nc	74.1
Carotenoids	35	58.8 (0, 141.5)	294.2 (0, 707.3)	Linear	b = 0.16 x = 58.83	0.16	74.1

[a] The correlation coefficient (r^2) is the adjusted r^2.

[b] nc refers to "not calculated" due to lack of a concentration-response or convergence of the model.

* Root number on day 35 at 0.3 mg/L PFOA was significantly higher than controls ($p < 0.05$). Longest root length on day 35 at 0.3 mg/L PFOA was significantly higher than controls ($p < 0.05$). The hormetic model did not converge with these data sets.

was <0.001% for all effect measure distributions (Figure 4). Based on current environmental concentrations of PFOA in the aquatic environment, HQ evaluations indicated that the likelihood of impacts on these plant species using either the threshold of toxicity or the lowest EC_{10} as the TBC is small (Table 7), even when concentrations would be abnormally high, such as under a spill condition (Moody *et al.* 2002). The toxicity threshold was found to be a more conservative measure of toxicity and hence estimation of risk than the lowest calculated EC_{10}. The ratios between the lowest *Myriophyllum* spp. EC_{10} and the corresponding toxicity threshold at days 14, 21, and 35 were 2.8, 3, and 4.6, respectively.

Discussion

Fluorinated surfactants, such as PFOA, are attracting increased attention due to their extreme persistence and global distribution. This study found that PFOA can induce toxicity in the aquatic plants *M. sibiricum* and *M. spicatum*, but only at relatively high concentrations. In the water column, the concentration of PFOA was stable, except at the greatest concentration, where partitioning into other compartments is suspected to account for some of the decline. Large filamentous algal bodies were observed to form in the microcosms with the greatest PFOA test concentration and may be partly responsible for the decline observed in PFOA concentrations due to bioconcentration into the algae, a phenomenon observed in rainbow trout (Martin *et al.* 2003). Still, PFOA may have partitioned into other matrices such as the sediment or the PVC liners, which was not investigated and should not be ruled out at this time. The recalcitrance of PFOA is not surprising considering that the same phenomena have been observed with perfluorooctane sulfonic acid (PFOS) (Boudreau *et al.* 2003b) and in smaller-chain fluorinated compounds such as trifluoroacetic acid (TFA) (Ellis *et al.* 2000) and chlorodifluoroacetic acid (Hanson *et al.* 2001) in aquatic microcosms.

Using two risk assessment methodologies to determine the likelihood of effects on *Myriophyllum* spp., we found negligible probability of effects occurring in these plants at current environment concentrations of PFOA. Only under spill conditions were concentrations of PFOA in the water great enough to expect an effect to occur, but such concentrations are not normally sustained long enough to be of concern to sessile,

Table 5. Effective concentrations required to cause a decrease in an endpoint by 10% and 50% from control (EC_{10}s, and EC_{50}s) as calculated using linear or nonlinear regression, with associated 95% confidence intervals, as well as the no observable effect concentrations (NOECs) for *Myriophyllum sibiricum* exposed to perfluorooctanoic acid as the sodium salt (PFOA) in aquatic microcosms

Endpoint	Time	EC_{10} (95% CI)	EC_{50} (95% CI)	Model	Parameters	r^{2a}	NOEC
Plant length (cm)	14	23.0 (0, 61.4)	51.7 (14.1, 89.3)	Logistic	a = 11.72 EC_{10} = 23.03 b = 7.72	0.61	74.1
Plant length (cm)	21	30.4 (0, 68.4)	50.0 (19.4, 80.6)	Logistic	a = 22.88 EC_{10} = 30.38 b = 4.41	0.88	23.9
Plant length (cm)	35	23.7 (5.3, 42.1)	41.3 (18.9, 63.7)	Logistic	a = 45.18 EC_{10} = 23.71 b = 3.96	0.85	23.9
Root number	14	20.9 (0, 46.8)	43.2 (12.4, 74.0)	Logistic	a = 8.17 EC_{10} = 20.90 b = 3.03	0.69	23.9
Root number	21	8.9 (6.0, 11.9)	44.6 (30.1, 59.3)	Linear	b = 13.01 EC_{10} = 8.93	0.72	23.9
Root number	35	29.2 (0, 69.2)	48.8 (14.8, 82.9)	Logistic	a = 18.50 EC_{10} = 29.21 b = 4.27	0.83	23.9
Root length (cm)	14	25.0 (0, 62.4)	40.9 (0, 87.3)	Logistic	a = 44, 18 EC_{10} = 25.00 b = 4.56	0.64	74.1
Root length (cm)	21	8.4 (5.2, 11.6)	42.9 (26.0, 58.2)	Linear	b = 86.86 EC_{10} = 8.42	0.65	23.9
Root length (cm)	35	24.8 (0, 64.1)	40.0 (0, 92.4)	Logistic	a = 177.9 EC_{10} = 24.78 b = 4.60	0.59	23.9
Longest root (cm)	14	9.0 (6.0, 11.9)	44.9 (30.2, 59.6)	Linear	b = 10.38 EC_{10} = 8.98	0.72	23.9
Longest root (cm)	21	25.7 (2.7, 48.7)	43.3 (18.4, 68.3)	Logistic	a = 14.75 EC_{10} = 25.70 b = 4.20	0.86	23.9
Longest root (cm)	35	30.0 (0, 90.6)	52.0 (5.2, 98.8)	Logistic	a = 17.45 EC_{10} = 29.97 b = 3.99	0.69	23.9
Node number	14	13.9 (2.1, 25.7)	69.6 (10.7, 128.6)	Linear	b = 6.15 EC_{10} = 13.93	0.30	74.1
Node number	21	37.8 (0, 175.5)	55.2 (0, 144.6)	Logistic	a = 10.92 EC_{10} = 37.83 b = 5.81	0.86	23.9
Node number	35	7.8 (6.0, 9.7)	39.1 (29.8, 48.4)	Linear	b = 20.04 EC_{10} = 7.82	0.82	23.9
Wet mass (g)	14	29.2 (0, 92.6)	45.6 (0, 104.5)	Logistic	a = 344.50 EC_{10} = 29.41 b = 5.02	0.69	74.1
Wet mass (g)	21	27.0 (0, 62.7)	70.4 (36.8, 104.1)	Logistic	a = 918.30 EC_{10} = 27.04 b = 7.28	0.82	23.9
Wet mass (g)	35	21.6 (0, 59.07)	37.9 (0, 241.0)	Gompertz	g = 3834.10 EC_{10} = 21.57 b = 3.34	0.68	23.9
Dry mass (g)	14	8.7 (2.7, 14.7)	43.5 (13.5, 73.4)	Linear	b = 14.80 EC_{10} = 8.70	0.36	74.1
Dry mass (g)	21	7.9 (5.0, 10.9)	39.6 (24.9, 54.4)	Linear	b = 50.79 EC_{10} = 7.93	0.66	74.1
Dry mass (g)	35	24.7 (0, 340.7)	35.8 (0, 76.2)	Gompertz	g = 241.30 EC_{10} = 24.67 b = 4.21	0.69	23.9
Chlorophyll-a	14	17.5 (1.2, 33.7)	87.3 (6.0, 168.5)	Linear	b = 0.38 EC_{10} = 17.45	0.27	74.1
Chlorophyll-a	21	17.2 (7.7, 26.7)	86.2 (38.7, 133.7)	Linear	b = 0.49 EC_{10} = 17.25	0.51	74.1
Chlorophyll-a	35	21.4 (0, 49.1)	106.9 (0, 245.3)	Linear	b = 0.36 EC_{10} = 21.39	0.16	74.1
Chlorophyll-b	14	26.7 (0, 73.4)	133.6 (0, 366.8)	Linear	b = 0.13 EC_{10} = 26.72	0.10	74.1
Chlorophyll-b	21	20.0 (1.7, 38.2)	99.9 (8.8, 191.0)	Linear	b = 0.19 EC_{10} = 19.98	0.28	74.1
Chlorophyll-b	35	nc[b]	nc	nc	nc	nc	74.1
Carotenoids	14	27.3 (0, 65.6)	136.3 (0, 327.8)	Linear	b = 0.14 EC_{10} = 27.26	0.14	74.1
Carotenoids	21	27.0 (5.8, 48.2)	135.1 (29.2, 241.1)	Linear	b = 0.16 EC_{10} = 27.03	0.57	74.1
Carotenoids	35	nc	nc	nc	nc	nc	74.1

[a]The correlation coefficient (r^2) is the adjusted r^2.
[b]nc refers to "not calculated" due to lack of a concentration-response or convergence.

rooted aquatic plants. At the second highest concentration of PFOA tested in this study, *Myriophyllum* spp. were able to grow and develop and some growth was observed at the highest concentration, although it was severely impaired. This implies that, if the toxicant was removed, this aquatic plant community could resume its normal growth pattern and potentially recover to control levels, even from a sustained spill or contamination event. The concentration of sodium used to neutralize the PFOA is likely not a factor in the observed toxicity in these plants, because similar concentrations have been used in previous studies, with no observed toxicity (Hanson *et al.* 2001).

The relative sensitivity of the two species of *Myriophyllum* spp. to PFOA was similar. Studies with the same plants and test systems, but examining PFOS as the potassium salt, found *M. sibiricum* to be more sensitive than *M. spicatum* to PFOS during the course of a 42-day exposure period (Hanson *et al.* 2004). In that study, toxicity was observed in the evaluated endpoints at >3.3 mg/L PFOS for EC_{10}s and >12.5 mg/L PFOS for EC_{50}s for *M. spicatum* and in *M. sibiricum* at >0.1 mg/L PFOS for EC_{10}s and >1.6 mg/L PFOS for EC_{50}s. The NOECs for *M. spicatum* were consistently ≥ 11.4 mg/L PFOS, while the NOECs for *M. sibiricum* were ≥ 0.3 mg/L PFOS. In general, PFOS was more toxic to *Myriophyllum* spp. than PFOA.

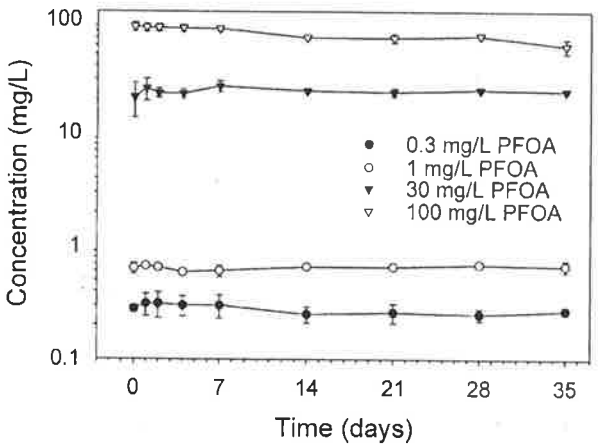

Fig. 1. The concentration of perfluorooctanoic acid (PFOA, as the sodium salt) over the course of the 35-day microcosm study.

The distinct difference in sensitivity between species exposed to PFOS was not observed in the current study with *Myriophyllum* spp. and PFOA. Endpoint sensitivity varied according to the duration of exposure, the level of effect chosen, and the plant species evaluated. This observation is

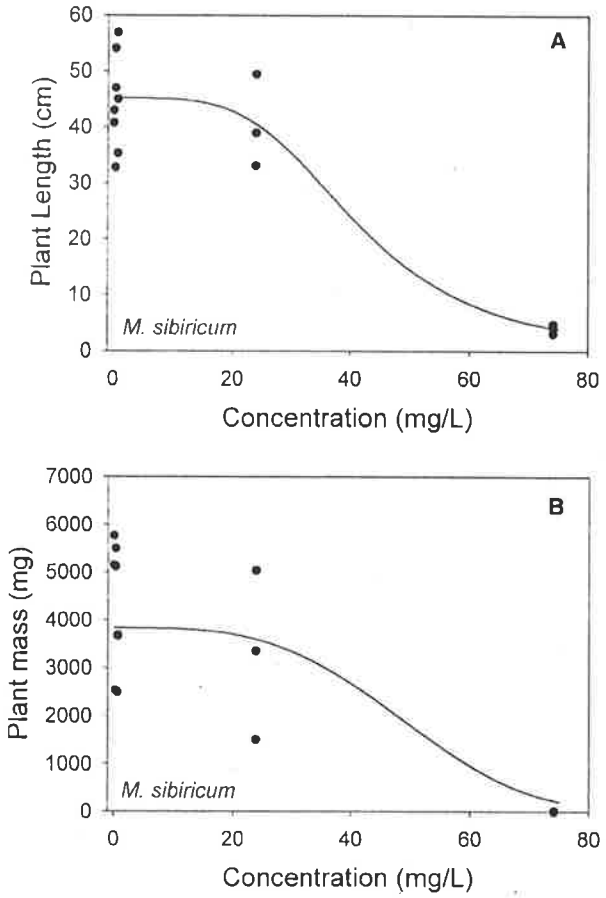

Fig. 2. The mean (n = 3) plant length (**A**) and wet mass (**B**) concentration–response for *Myriophyllum sibiricum* after 35 days of exposure to perfluorooctanoic acid (PFOA, as the sodium salt). Curves were fitted using a logistic model.

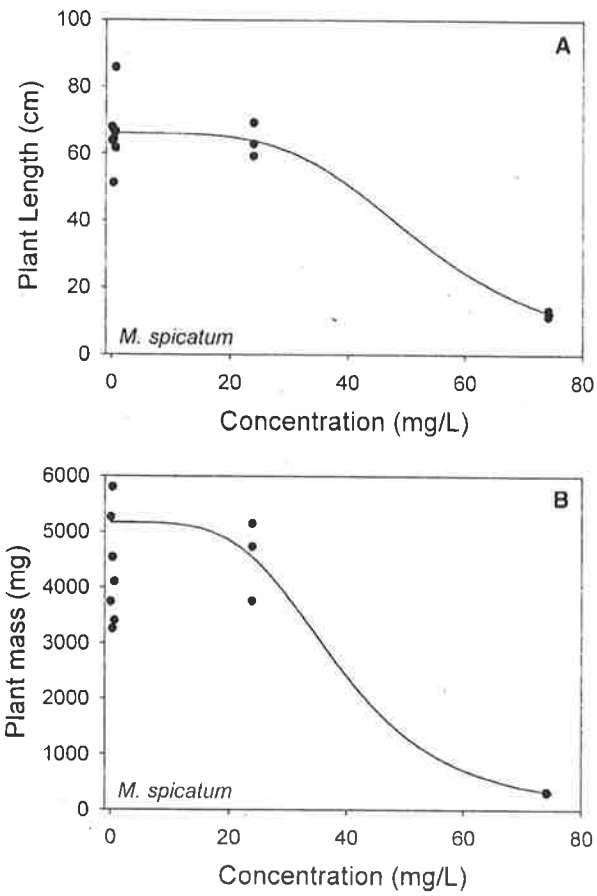

Fig. 3. The mean (n = 3) plant length (**A**) and wet mass (**B**) concentration–response for *Myriophyllum spicatum* after 35 days of exposure to perfluorooctanoic acid (PFOA, as the sodium salt). Curves were fitted using a logistic model.

consistent with other field and laboratory studies with these plants (Hanson *et al.* 2002; Hanson and Solomon 2004a; Hanson *et al.* 2004) and supports the argument that a suite of endpoints should be assessed when these macrophytes are used in ecotoxicological studies. Considering that the mechanism of action of this compound in plants is not known, other endpoints, such as chlorophyll fluorescence parameters (Marwood *et al.* 2003), may be more sensitive indicators of PFOA toxicity. The NOEC was found to be, on average, a less conservative measure of response than the EC_{10} for the same endpoints. The EC_{10} in turn was anywhere from three- to fivefold greater than the toxicity threshold, when the smallest calculated values were compared at each date and for both plant species. The toxicity threshold estimated from an effect measure distribution is a more conservative estimate of response than either the EC_{10} or the NOEC for these plants. These distributions have been used effectively with field and laboratory data (Hanson and Solomon 2002, 2004b; Hanson *et al.* 2004) and may provide a method to more accurately estimate the toxicological risk that a compound poses to an aquatic plant.

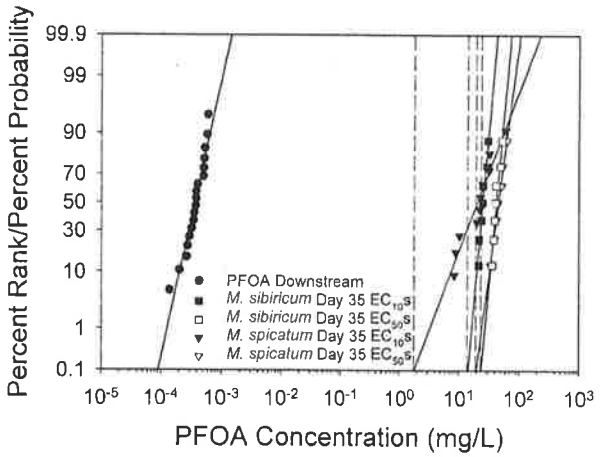

Fig. 4. Effect measure distributions for *Myriophyllum spicatum* and *M. sibiricum* exposed to perfluorooctanoic acid (PFOA, as the sodium salt) at the EC_{10} and EC_{50} levels for day 35 with the PFOA exposure distribution for the Tennessee River, USA. The dashed line represents the toxicity threshold for that specific distribution.

Table 6. Regression coefficients, intercepts, and thresholds of toxicity for *Myriophyllum sibiricum* and *M. spicatum* toxicity from exposure to perfluorooctanoic acid (PFOA) as the sodium salt in aquatic microcosms using the Weibull equation to create effect measure distributions

	$y = ax + b$[a]			Regression intercepts (mg/L)[b]			
Distribution	a	b	r^2	Toxicity threshold	10th/90th centile	n[c]	W-test[d] ($p > 0.05$)
M. spicatum Day 14 EC_{10}	3.84	−5.24	0.92	3.6	10.7	7	Pass
M. spicatum Day 21 EC_{10}	4.16	−4.56	0.90	2.3	6.1	7	Pass
M. spicatum Day 35 EC_{10}	2.94	−3.83	0.93	1.8	7.43	10	Pass
M. spicatum Day 14 EC_{50}	nc[e]	nc	nc	nc	nc	nc	nc
M. spicatum Day 21 EC_{50}	5.27	−8.91	0.97	12.7	28.0	7	Pass
M. spicatum Day 35 EC_{50}	8.58	−14.23	0.99	19.9	32.3	7	Pass
M. sibiricum Day 14 EC_{10}	3.99	−5.06	0.88	3.1	8.9	10	Pass
M. sibiricum Day 21 EC_{10}	3.11	−3.93	0.89	1.9	7.1	10	Pass
M. sibiricum Day 35 EC_{10}	3.50	−4.66	0.65	2.8	9.2	8	Fail
M. sibiricum Day 35 EC_{10}*	13.20	−18.42	0.92	14.5	19.9	7	Pass
M. sibiricum Day 14 EC_{50}	8.74	−14.68	0.76	21.2	34.1	7	Fail
M. sibiricum Day 21 EC_{50}	8.69	−14.65	0.88	21.4	34.5	7	Pass
M. sibiricum Day 35 EC_{50}	12.71	−20.61	0.91	23.9	33.2	7	Pass
Myriophyllum spp. Day 14 EC_{10}	4.38	−5.73	0.95	4.0	10.4	17	Pass
Myriophyllum spp. Day 21 EC_{10}	3.71	−4.43	0.98	2.3	7.1	17	Pass
Myriophyllum spp. Day 35 EC_{10}	3.17	−4.55	0.90	2.9	10.7	17	Pass
Myriophyllum spp. Day 14 EC_{50}	8.36	−14.18	0.84	21.2	34.9	8	Fail
Myriophyllum spp. Day 21 EC_{50}	7.26	−12.25	0.95	18.3	32.4	14	Pass
Myriophyllum spp. Day 35 EC_{50}	11.02	−18.06	0.95	22.8	33.3	14	Pass
Tennessee River Downstream	17.64	5.13	0.93	nc	0.61	18	nc

Note: An exposure distribution for PFOA was modeled from concentrations found in the Tennessee River downstream of a fluorochemical manufacturing facility (Hansen *et al.* 2002).

[a] These values are transformed into units of log and probit for the purposes of regression, and backtransforms were used to calculate the intercepts. The distribution units were in mg/L.

[b] Toxicity threshold is calculated from the 0.1 centile from the effect measure distributions. The 10th centile is calculated for the effect measure distributions, and the 90th centile is calculated for the PFOA exposure distributions.

[c] Number of data points used in the ranking.

[d] The W-test is the Shapiro-Wilk test for normality ($p > 0.05$).

[e] nc refers to not calculated.

* The lowest effect measure was removed due to its skewing effect on this distribution.

Table 7. The hazard quotients (HQ) calculated from various environmental concentrations in freshwater and the lowest thresholds of toxicity and EC_{10}s calculated from toxicity data for *Myrioyhyllum spicatum* and *M. sibiricum* exposed to perfluorooctanoic acid as the sodium salt (PFOA)

			Day 14 HQ		Day 21 HQ		Day 35 HQ	
PFOA (mg/L)	Location	Reference	Toxicity threshold	EC_{10}	Toxicity threshold	EC_{10}	Toxicity threshold	EC_{10}
0.105	Wurtsmith Airforce Base, USA	Moody *et al.* 2003	0.34	0.12	0.55	0.18	0.58	0.13
0.0113	Etobicoke Creek, Canada	Moody *et al.* 2002[a]	<0.04	<0.02	<0.06	<0.02	<0.06	<0.02
0.00002	Etobicoke Creek, Canada	Moody *et al.* 2002[b]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
0.000598	Tennessee River, USA	Hansen *et al.* 2002	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a] The concentration of PFOA 2 days after a spill event of fluorinated surfactant firefighting foam.

[b] The concentration of PFOA several weeks after a spill event of fluorinated surfactant firefighting foam.

The results of this study indicate that PFOA does not appear to pose a significant risk to the growth and development of *Myriophyllum* spp. or aquatic macrophytes at the concentrations commonly quantified in the aquatic environment. Still, due to its persistence, PFOA warrants continued environmental monitoring and further testing of other aquatic organisms, specifically algae, which tend to be more sensitive to fluorinated compounds than other aquatic species (Boutonnet *et al.* 1999).

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